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Electronically Controlled Stereochemistry in the Reaction of Chiral NAD(P) +/NAD(P)H Analogs (BIOORGANIC CHEMISTRY - Bioorganic Reaction Theory)

AUTHOR(S):

Ohno, Atsuyoshi; Tsutsumi, Akihiro; Kawai, Yasushi; Yamazaki, Norimasa

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Electronically Controlled Stereochemistry in the Reaction of Chiral $\text{NAD(P)}^+/\text{NAD(P)H}$ Analogs

Atsuyoshi Ohno, Akihiro Tsutsumi, Yasushi Kawai and Norimasa Yamazaki

The *N*-methylpyridinium salt of 6, 7-dihydro-6-methyl-5-oxopyridino[3, 2-*d*]-2-benzazepin has been synthesized. The salt has axial chirality with respect to the orientation of the carbonyl dipole. An enantiomer of the cation has been obtained as the iodide salt. Reduction of the salt results in the corresponding dihydropyridine derivative stereospecifically. The stereochemistry of the reduction is controlled entirely by the electronic effect of the carbonyl dipole.

Keywords: $\text{NAD(P)}^+/\text{NAD(P)H}$ model compound/ Axial chirality/ Stereochemistry

Although pyridinium/dihydropyridine moieties in $\text{NAD(P)}^+/\text{NAD(P)H}$ -coenzymes are achiral, *re*- and *si*-faces of the molecules are recognized by a substrate when they are set in a pocket of enzyme. Some oxidoreductases prefer the attack from the *re*-face, while the others react in the *si*-face. From the viewpoint of chemical evolution of an enzyme, the difference in stereochemistry as well as mechanistic trick is an interesting subject. There are two possibilities in stereochemical evolution of oxidoreductase to the present forms: functional and random [1].

We reported homogeneous reaction systems where the stereochemistry of chiral $\text{NAD(P)}^+/\text{NAD(P)H}$ analogs ($\text{Me}_3\text{PNP}^+/\text{Me}_3\text{PNPH}$ and $\text{Me}_3\text{MQP}^+/\text{Me}_3\text{MQPH}$), in which the stereochemical course of the redox reaction is influenced by the orientation of a carbonyl group, is controlled by the reactivity of a substrate [2], in contrast to the conclusion presented by Brounts and Buck based on quantum mechanical calculation. As these authors have mentioned, the substrate assigned for the calculation

carries a positive charge, and the charge-dipole interaction appears to be important in these calculations when the carbonyl dipole is *syn* to the reacting hydrogen. Since the stereochemistry observed in these organic systems is exactly parallel to those of enzymatic systems classified by Nambiar *et al.* [3], we studied the mechanism for stereochemical control in this and similar systems extensively and came to a conclusion that the interaction at the ground state is quite important [4].

In order to obtain closer analog of $\text{NAD(P)}^+/\text{NAD(P)H}$ coenzymes for testing the orientational effect of the carbonyl group more directly, we synthesized an *N*-methylpyridinium salt of 6, 7-dihydro-6-methyl-5-oxopyridino [3, 2-*d*]-2-benzazepin (MeMPA^+) and its dihydropyridine derivative (MeMPAH). Unfortunately, conformational stability of MeMPA^+I^- is not sufficient, and the optically active enantiomer of this salt racemizes at room temperature easily.

The ^1H NMR spectrum of MeMPAH in CD_3CN shows completely separated signals arising from two methylene

BIOORGANIC CHEMISTRY —Physical Bioorganic Chemistry—

Scope of research

Biochemical reactions are studied from the viewpoint of physical organic chemistry. Namely, the reaction mechanism and stereochemistry of *NAD*-dependent oxidoreductases are explored. Stereospecific redox transformations mediated by certain biocatalysts such as microbes, enzymes, cultured tissues are also studied. The results will be applied to develop new organic reactions.



Professor
OHNO, Atsuyoshi
(D Sc)



Associate Professor
NAKAMURA, Kaoru
(D Sc)



Instructor
SUGIYAMA, Takashi



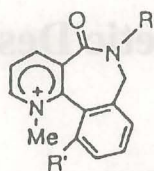
Instructor
KAWAI, Yasushi
(D Sc)

Technician:

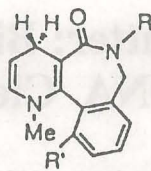
HIRANO, Toshiko

Students:

KAWASAKI, Masashi (RF)
KONDO, Shin-ichi (DC)
TSUTSUMI, Akihiro (DC)
KINOSHITA, Masamichi (DC)
SHIOJI, Kosei (DC)
YAMAZAKI, Norimasa (DC)
KUNITOMO, Jun (DC)
TAKAHASHI, Akiko (DC)
INOUE, Yuko (MC)
SAITOU, Kentarou (MC)
NAKAGAWA, Toshiya (MC)
HIDA, Kouichi (MC)
KUBO, Yuji (RS)
TODA, Toshiyuki (RS)
MATSUDA, Tomoko (RS)
YAMAGUCHI, Satoshi (RS)



R=Me, R'=H: MeMPA⁺
R=tBu, R'=H: BuMPA⁺
R=Me, R'=Me: 3Me-MeMPA⁺



R=Me, R'=H: MeMPAH
R=tBu, R'=H: BuMPAH
R=Me, R'=Me: 3Me-MeMPAH

mprotons at the C₄-position (*syn* and *anti* to the carbonyl oxygen). Therefore, the stereochemistry associated with the reduction of MeMPA⁺ with a deuterated reagent can be monitored quite easily. The results are summarized in Table 1 together with those of BuMPA⁺. It is interesting to note that a (net) hydride originating from BNAH or its analog attacks MeMPA⁺ from the side of the pyridinium ring where the carbonyl oxygen lies, even though this is the more sterically hindered face. Thus, the stereochemistry of the reaction cannot be explained as a steric effect, and there is no doubt that the carbonyl dipole or an electronic effect plays an important role in determining the stereochemical outcome of the reaction. The stereochemical result of the reduction with sodium dithionite in D₂O affords a *syn/anti* ratio of 50/50, which is different from those with BNAH and its analogs. However, we must point out the possibility that the compound has undergone racemization during the processes of isolation and spectroscopy. Indeed, a preliminary result from the reduction of 6,7-dihydro-6-methyl-5-oxopyridino[3,2-*d*]-2-(3-methylbenz) azepin (3Me-MeMPA⁺), the conformation of which is stable at

Table 1. Reduction of Racemic Mixture of MeMPA⁺ and BuMPA⁺ ^a

NAD ⁺ -Analog	Time h	Reducing Reagent	Stereochemistry ^{b,c} <i>syn</i> : <i>anti</i>
MeMPA ⁺ (R=Me)	1.0	Na ₂ S ₂ O ₄ /D ₂ O	50 : 50
	39 ^d	BNAH-4, 4- <i>d</i> ₂	65 : 35
	1.5	(<i>R</i>)-Me ₂ PNPH-4- <i>d</i>	58 : 42
	1.5	(<i>S</i>)-Me ₂ PNPH-4- <i>d</i>	60 : 40
BuMPA ⁺ (R=tBu)	1.0	Na ₂ S ₂ O ₄ /D ₂ O	51 : 49
	1.5	(<i>R</i>)-Me ₂ PNPH-4- <i>d</i>	70 : 30
	1.5	(<i>S</i>)-Me ₂ PNPH-4- <i>d</i>	69 : 31

a: About 20% of MeMPAD or BuMPAD produced reacts with MeMPA⁺ or BuMPA⁺, respectively, yielding 4,4-dihydro and 4,4-dideuterio compounds. b: Relative to the carbonyl oxygen. c: Estimated error is about ±3 for all numbers. d: Reaction at 35°C.

room temperature, in contrast to the unstability of those MeMPA⁺ and BuMPA⁺, with sodium dithionite has revealed that the *syn/anti* ratio is 80/20. Further investigation is necessary before a conclusion is formed on the stereochemical difference between hot and cold reducing agents.

It has been proposed that the carbamoyl moiety in an NADH analog faces a polar side chain of the substrate or oxidizing agent at the transition state of a homogeneous reaction [5]. Magnesium ion promotes this face-to-face interaction by coordinating on itself both reducing and oxidizing agents. Not only is the stereochemistry improved by the sandwich-like interaction of magnesium ion, but the reaction rate is also increased by its catalytic effect. The present reaction, however, is retarded by the presence of magnesium ion, which is quite reasonable because one of the agents is an onium, and it is highly plausible that a cation is hardly coordinated on a cationic magnesium ion. Thus, a binary complex between the reducing and oxidizing agents is a plausible intermediate in the present reaction even in the presence of magnesium ion.

The fact that both (4*R*)- and (4*S*)-Me₂PNPD afford the same *syn/anti* ratio within experimental error confirms the idea that face-to-face interaction between the carbonyl group in the onium and the one in the reducing agent is important at the transition state of the reaction as proposed previously.

To our best knowledge, MeMPA⁺/MeMPAH system is the first example of molecular asymmetry stemming from the orientation of the carbonyl group only and resolved to each enantiomer.

The present result strongly supports the possibility of functional model for chemical evolution of an enzyme, where it is predicted that NAD(P)⁺/NAD(P)H coenzymes themselves can induce chirality into an achiral substrate during a redox reaction without stereochemical assistance of a protein [6].

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